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(21) International Application Number: PCT/US97/04755 (22) International Filing Date: 21 March 1997 (21.03.97) (30) Priority Data: 08/590,554 21 March 1996 (21.03.96) US (71) Applicant: MYCOGEN CORPORATION [US/US]; 5501 Oberlin Drive, San Diego, CA 92121 (US). (72) Inventors: PAYNE, Jewel; 2318 Elendil Lane, Davis, CA 95616 (US). NARVA, Kenneth, E.; 12123 Caminito Mira Del Mar, San Diego, CA 92130 (US). FU, Jenny; 4533 Du Vinci Street, San Diego, CA 92130 (US). (74) Agents: SANDERS, Jay, M. et al.; Saliwanchik, Lloyd & Saliwanchik, Suite A-1, 2421 N.W. 41st Street, Gainesville, FL 32606 (US).		(81) Designated States: AU, BR, CA, JP, KR, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: BACILLUS THURINGIENSIS GENES ENCODING NEMATODE-ACTIVE TOXINS (57) Abstract <p><i>B. thuringiensis</i> genes encoding toxins active against nematode pests have been cloned from the strains PS80J11, PS158D5, PS167P, PS169E, PS177F1, PS177G, PS204G4 and PS204G6. The toxins are active especially against <i>Panagrellus redivivus</i> as exemplified in the application. The DNAs encoding the toxins can be used to transform various hosts, such as plants, to express the toxins.</p>		

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DESCRIPTION**BACILLUS THURINGIENSIS GENES ENCODING NEMATODE-ACTIVE TOXINS**

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Cross-Reference to Related Applications

This application is a continuation-in-part of co-pending application Serial No. 08/485,568, filed June 7, 1995; which is a continuation-in-part of application Serial No. 08/310,197, filed September 21, 1994; which is a division of Serial No. 08/092,155, filed July 15, 1993, now U.S. Patent No. 5,350,577; which is a division of Serial No. 07/918,345, filed July 21, 1992, now U.S. Patent No. 5,270,448; which is a division of 07/558,738, filed July 27, 1990, now U.S. Patent No. 5,151,363. This application is also a continuation-in-part of co-pending application Serial No. 08/357,698, filed December 16, 1994; which is a division of Serial No. 08/176,403, filed December 30, 1993, now abandoned; which is a continuation-in-part of 07/999,053, filed December 31, 1992, now abandoned.

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Background of the Invention

The soil microbe *Bacillus thuringiensis* (*B.t.*) is a Gram-positive, spore-forming bacterium characterized by parasporal crystalline protein inclusions. These inclusions often appear microscopically as distinctively shaped crystals. The proteins can be highly toxic to pests and specific in their toxic activity. Certain *B.t.* toxin genes have been isolated and sequenced, and recombinant DNA-based *B.t.* products have been produced and approved for use. In addition, with the use of genetic engineering techniques, new approaches for delivering *B.t.* endotoxins to agricultural environments are under development, including the use of plants genetically engineered with endotoxin genes for insect resistance and the use of stabilized intact microbial cells as *B.t.* endotoxin delivery vehicles (Gaertner and Kim, 1988). Thus, isolated *B.t.* endotoxin genes are becoming commercially valuable.

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Until the last ten years, commercial use of *B.t.* pesticides has been largely restricted to a narrow range of lepidopteran (caterpillar) pests. Preparations of the spores and crystals of *B. thuringiensis* var. *kurstaki* have been used for many years as commercial insecticides for lepidopteran pests. For example, *B. thuringiensis* var. *kurstaki* HD-1 produces a crystal called a δ -endotoxin which is toxic to the larvae of a number of lepidopteran insects.

In recent years, however, investigators have discovered *B.t.* pesticides with specificities for a much broader range of pests. For example, other species of *B.t.*, namely *B.t.* var.

israelensis and *B.t.* var. *tenebrionis* (a.k.a. M-7, a.k.a. *B.t.* var. *san diego*), have been used commercially to control insects of the orders Diptera and Coleoptera, respectively (Gaertner, 1989). See also Couch, 1980 and Beegle, 1978. Krieg *et al.*, 1983, describe *Bacillus thuringiensis* var. *tenebrionis*, which is reportedly active against two beetles in the order Coleoptera. These are the Colorado potato beetle, *Leptinotarsa decemlineata*, and the beetle *Agelastica alni*.

Recently, new subspecies of *B.t.* have been identified, and genes responsible for active δ -endotoxin proteins have been isolated (Höfte and Whiteley, 1989). Höfte and Whiteley classified *B.t.* crystal protein genes into 4 major classes. The classes were CryI (Lepidoptera-specific), CryII (Lepidoptera- and Diptera-specific), CryIII (Coleoptera-specific), and CryIV (Diptera-specific). Prefontaine *et al.*, 1987, describe probes useful in classifying lepidopteran-active genes. The discovery of strains specifically toxic to other pests has been reported (Feitelson *et al.*, 1992).

The cloning and expression of a *B.t.* crystal protein gene in *Escherichia coli* has been described in the published literature (Schnepf and Whiteley, 1981). U.S. Patent 4,448,885 and U.S. Patent 4,467,036 both disclose the expression of *B.t.* crystal proteins in *E. coli*. U.S. Patents 4,797,276 and 4,853,331 disclose *B. thuringiensis* var. *tenebrionis* (a.k.a. *B.t. san diego*, a.k.a. M-7) which can be used to control coleopteran pests in various environments. U.S. Patent No. 4,918,006 discloses *Bacillus thuringiensis* var. *israelensis* toxins which are active against dipteran pests. This patent reports that a protein of about 27 kD, and fragments thereof, are responsible for the dipteran activity. U.S. Patent No. 4,849,217 discloses *B.t.* isolates which have activity against the alfalfa weevil. U.S. Patent No. 5,151,363 and U.S. Patent No. 4,948,734 disclose certain isolates of *B.t.* which have activity against nematodes.

The accepted methodology for control of nematodes has centered around the use of the drug benzimidazole and its congeners. The use of these drugs on a wide scale has led to many instances of resistance among nematode populations (Prichard *et al.*, 1980; Coles, 1986). There are more than 100,000 described species of nematodes.

A small number of research articles have been published concerning the effects of δ -endotoxins from *B. thuringiensis* species on the viability of nematode eggs. Bottjer *et al.* (1985) have reported that *B.t. kurstaki* and *B.t. israelensis* were toxic *in vitro* to eggs of the nematode *Trichostrongylus colubriformis*. In addition, 28 other *B.t.* strains were tested with widely variable toxicities. The most potent had LD₅₀ values in the nanogram range. Ignoffo and Dropkin (1977) have reported that the thermostable toxin from *Bacillus thuringiensis* (beta exotoxin) was active against a free-living nematode, *Panagrellus redivivus* (Goodey); a plant-

parasitic nematode, *Meloidogyne incognita* (Chitwood); and a fungus-feeding nematode, *Aphelenchus avena* (Bastien). Beta exotoxin is a generalized cytotoxic agent with little or no specificity. Also, Ciordia and Bizzell (1961) gave a preliminary report on the effects of *B. thuringiensis* on some cattle nematodes.

At the present time there is a need to have more effective means to control the many nematodes that cause considerable damage to susceptible hosts. Effective means would advantageously employ biological agents, such as *B.t.* pesticides. As a result of extensive research and investment of resources, many other patents have issued for new *B.t.* isolates and new uses of *B.t.* isolates. However, the discovery of new *B.t.* isolates and new uses of known *B.t.* isolates remains an empirical, unpredictable art.

Brief Summary of the Invention

The subject invention concerns novel δ -endotoxin genes obtainable from *B.t.* isolates PS167P, PS80JJ1, PS158D5, PS169E, PS177F1, PS177G, PS204G4, and PS204G6, wherein the genes encode proteins which are active against nematode pests. These toxin genes can be transferred to suitable hosts as described herein.

Further aspects of the subject invention concern nematode-active toxins, and fragments thereof, encoded by the genes disclosed herein. Another embodiment of the subject invention concerns hosts transformed with the genes of the subject invention. In a preferred embodiment, the transformed hosts are plants.

Brief Description of the Drawings

Figure 1 is a photograph of 9% SDS polyacrylamide gel electrophoresis showing alkali-soluble proteins of nematode active strains.

Gel A: Lane (1) Protein standard, (2) PS17, (3) PS33F2, (4) PS52A1, (5) PS63B, (6), PS69D1, (7) PS80JJ1, (8) PS177F1, (9) PS177G, (10) PS204G6, (11) Protein standard.

Gel B: Lane (1) Protein standard, (2) PS17, (3) PS33F2, (4) PS52A1, (5) PS63B, (6), PS69D1, (7) PS169E, (8) PS167P, (9) PS204G4, (10) PS158D5, (11) Protein standard.

Brief Description of the Sequences

SEQ ID NO. 1 is the nucleotide sequence of a "forward" oligonucleotide primer used for PCR amplification of the 80JJ1 and 167P genes.

SEQ ID NO. 2 is the nucleotide sequence of a "reverse" oligonucleotide primer used for PCR amplification of the 80JJ1 and 167P genes.

SEQ ID NO. 3 is the nucleotide sequence of the 80JJ1 toxin gene.

SEQ ID NO. 4 is the amino acid sequence of the 80JJ1 protein.

SEQ ID NO. 5 is the nucleotide sequence of the 167P toxin gene.

SEQ ID NO. 6 is the amino acid sequence of the 167P protein.

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Detailed Disclosure of the Invention

The subject invention pertains to novel genes which encode nematode-active toxins. The toxins themselves are also an important aspect of the invention. A further embodiment of the subject invention is the transformation of suitable hosts to confer upon these hosts the ability to express nematode-active toxins.

The *Bacillus thuringiensis* isolates from which the genes of the subject invention can be obtained have been deposited in the permanent collection of the Agricultural Research Service Patent Culture Collection (NRRL), Northern Regional Research Center, 1915 North University Street, Peoria, Illinois 61604, USA. The accession numbers are as follows:

15	<u>Culture</u>	<u>Repository No.</u>	<u>Deposit Date</u>
	<i>B.t.</i> strain PS80JJ1	NRRL B-18679	July 17, 1990
	<i>B.t.</i> strain PS158D5	NRRL B-18680	July 17, 1990
	<i>B.t.</i> strain PS167P	NRRL B-18681	July 17, 1990
	<i>B.t.</i> strain PS169E	NRRL B-18682	July 17, 1990
20	<i>B.t.</i> strain PS177F1	NRRL B-18683	July 17, 1990
	<i>B.t.</i> strain PS177G	NRRL B-18684	July 17, 1990
	<i>B.t.</i> strain PS204G4	NRRL B-18685	July 17, 1990
	<i>B.t.</i> strain PS204G6	NRRL B-18686	July 17, 1990
	<i>E. coli</i> NMS22(pMYC2379)	NRRL B-21155	November 3, 1993
25	<i>E. coli</i> NMS22(pMYC2382)	NRRL B-21329	September 28, 1994

The subject cultures have been deposited under conditions that assure that access to the cultures will be available during the pendency of this patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 CFR 1.14 and 35 USC 122. The deposits are available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of the deposits does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

Further, the subject culture deposits will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., they

will be stored with all the care necessary to keep them viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of a deposit, and in any case, for a period of at least 30 (thirty) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the cultures. The depositor
5 acknowledges the duty to replace the deposit(s) should the depository be unable to furnish a sample when requested, due to the condition of the deposit(s). All restrictions on the availability to the public of the subject culture deposits will be irrevocably removed upon the granting of a patent disclosing them.

Genes and toxins. The genes and toxins useful according to the subject invention
10 include not only the full length sequences disclosed but also fragments of these sequences, variants, mutants, and fusion proteins which retain the characteristic pesticidal activity of the toxins specifically exemplified. In some instances, the fusion protein may contain, in addition to the characteristic pesticidal activity of the toxins specifically exemplified, another pesticidal activity contributed by the fusion process. As used herein, the terms "variants" or "variations"
15 of genes refer to nucleotide sequences which encode the same toxins or which encode equivalent toxins having similar pesticidal activity. As used herein, the term "equivalent toxins" refers to toxins having the same or essentially the same biological activity against the target pests as the claimed toxins.

It should be apparent to a person skilled in this art that genes encoding nematode-active
20 toxins can be identified and obtained through several means. The specific genes exemplified herein may be obtained from the isolates deposited at a culture depository as described above. These genes, or portions or variants thereof, may also be constructed synthetically, for example, by use of a gene synthesizer. Variations of genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using
25 commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as *Bal31* or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which encode active fragments may be obtained using a variety of restriction enzymes. Proteases may be used to directly obtain active fragments of these toxins.

30 Equivalent toxins and/or genes encoding these equivalent toxins can be derived from *B.t.* isolates and/or DNA libraries using the teachings provided herein. There are a number of methods for obtaining the pesticidal toxins of the instant invention. For example, antibodies to the pesticidal toxins disclosed and claimed herein can be used to identify and isolate other toxins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the toxins

which are most constant and most distinct from other *B.t.* toxins. These antibodies can then be used to specifically identify equivalent toxins with the characteristic activity by immunoprecipitation, enzyme linked immunosorbent assay (ELISA), or Western blotting. Antibodies to the toxins disclosed herein, or to equivalent toxins, or fragments of these toxins, can readily be prepared using standard procedures in this art. The genes which encode these toxins can then be obtained from the microorganism.

Fragments and equivalents which retain the pesticidal activity of the exemplified toxins would be within the scope of the subject invention. Also, because of the redundancy of the genetic code, a variety of different DNA sequences can encode the amino acid sequences disclosed herein. It is well within the skill of a person trained in the art to create these alternative DNA sequences encoding the same, or essentially the same, toxins. These variant DNA sequences are within the scope of the subject invention. As used herein, reference to "essentially the same" amino acid sequence refers to sequences which have amino acid substitutions, deletions, additions, or insertions which do not materially affect the pesticidal activity of the protein.

A further method for identifying the toxins and genes of the subject invention is through the use of oligonucleotide probes. These probes are nucleotide sequences having a means for detection. As is well known in the art, if the probe molecule and nucleic acid sample hybridize by forming a strong bond between the two molecules, it can be reasonably assumed that the probe and sample have substantial homology. The probe's means of detection provides a means for determining in a known manner whether hybridization has occurred. Such a probe analysis provides a rapid method for identifying toxin-encoding genes of the subject invention. The nucleotide segments which are used as probes according to the invention can be synthesized by use of DNA synthesizers using standard procedures. These nucleotide sequences can also be used as PCR primers to amplify genes of the subject invention.

Certain toxins of the subject invention have been specifically exemplified herein. Since these toxins are merely exemplary of the toxins of the subject invention, it should be readily apparent that the subject invention further comprises variant or equivalent toxins (and nucleotide sequences coding for equivalent toxins) having the same or essentially the same pesticidal activity of the exemplified toxins. These equivalent toxins can have amino acid homology with an exemplified toxin. This amino acid homology will typically be greater than 75%, preferably be greater than 90%, and most preferably be greater than 95%. The amino acid homology will be highest in certain critical regions of the toxin which account for biological activity or are involved in the determination of three-dimensional configuration which ultimately is responsible

for the biological activity. In this regard, certain amino acid substitutions are acceptable and can be expected if these substitutions are in regions which are not critical to activity or are conservative amino acid substitutions which do not affect the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Table 1 provides a listing of examples of amino acids belonging to each class.

Table 1

Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not significantly detract from the biological activity of the toxin.

The toxins of the subject invention can also be characterized in terms of the shape and location of toxin inclusions.

Following is a table which provides characteristics of certain isolates useful according to the subject invention.

Table 2. Description of *B.t.* strains toxic to nematodes

Culture	Crystal Description	Approx. MW (kDa)	Serotype	NRRL Deposit	Deposit Date
PS80JJ1	multiple attached	130, 90, 47, 37	4a4b, sotto	B-18679	7-17-90
PS158D5	attached amorphous	80	novel	B-18680	7-17-90
PS167P	attached amorphous	120	novel	B-18681	7-17-90
PS169E	attached amorphous	150, 128, 33	non-motile	B-18682	7-17-90
PS177F1	multiple attached	140, 116, 103, 62	non-motile	B-18683	7-17-90
PS177G	multiple attached	135, 125, 107, 98, 62	non-motile	B-18684	7-17-90
PS204G4	multiple attached	105, 98, 90, 60, 44, 37	non-motile	B-18685	7-17-90
PS204G6	long amorphous	23, 21	wuhanensis	B-18686	7-17-90

N.D. = not determined

Recombinant hosts. The toxin-encoding genes harbored by the isolates of the subject invention can be introduced into a wide variety of microbial or plant hosts. Expression of the toxin gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide. With suitable microbial hosts, e.g., *Pseudomonas*, the microbes can be applied to the situs of the pest, where they will proliferate and be ingested by the pest. The result is control of the pest. Alternatively, the microbe hosting the toxin gene can be treated under conditions that prolong the activity of the toxin and stabilize the cell. The treated cell, which retains the toxic activity, then can be applied to the environment of the target pest.

Where the *B.t.* toxin gene is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, it is advantageous to use certain host microbes. For example, microorganism hosts can be selected which are known to occupy the pest's habitat. Microorganism hosts may also live symbiotically with a specific species of pest. These microorganisms are selected so as to be capable of successfully competing in the particular environment with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for improved protection of the pesticide from environmental degradation and inactivation.

A large number of microorganisms are known to inhabit the habitat of pests. These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, e.g., genera *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*, *Xanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylophilus*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*, *Arthrobacter*, *Azotobacter*, *Leuconostoc*, and *Alcaligenes*; fungi, e.g., genera *Metarhizium*, *Bavaria*, *Saccharomyces*, *Cryptococcus*, *Kluyveromyces*, *Sporobolomyces*, *Rhodotorula*, and *Aureobasidium*. Of particular interest are such bacterial species as *Pseudomonas syringae*, *Pseudomonas fluorescens*, *Serratia marcescens*, *Acetobacter xylinum*, *Agrobacterium tumefaciens*, *Rhodopseudomonas spheroides*, *Xanthomonas campestris*, *Rhizobium melioli*, *Alcaligenes entrophus*, and *Azotobacter vinlandii*; and yeast species such as *Rhodotorula rubra*, *R. glutinis*, *R. marina*, *R. aurantiaca*, *Cryptococcus albidus*, *C. diffluens*, *C. laurentii*, *Saccharomyces rosei*, *S. pretoriensis*, *S. cerevisiae*, *Sporobolomyces roseus*, *S. odoratus*, *Kluyveromyces veronae*, and *Aureobasidium pollulans*. Of particular interest are the pigmented microorganisms.

A wide variety of ways are available for introducing a *B.t.* gene encoding a toxin into a microorganism host under conditions which allow for stable maintenance and expression of the gene. These methods are well known to those skilled in the art and are described, for example, in United States Patent No. 5,135,867, which is incorporated herein by reference.

Control of nematodes using the isolates, toxins, and genes of the subject invention can be accomplished by a variety of methods known to those skilled in the art. These methods include, for example, the application of *B.t.* isolates to the pests (or their location), the application of recombinant microbes to the pests (or their locations), and the transformation of plants with genes which encode the pesticidal toxins of the subject invention. Recombinant microbes may be, for example, a *B.t.*, *E. coli*, or *Pseudomonas*. Transformations can be made by those skilled in the art using standard techniques. Materials necessary for these transformations are disclosed herein or are otherwise readily available to the skilled artisan. For example, the gene encoding the 167P toxin is provided herein as SEQ ID NO. 5. The deduced amino acid sequence for the 167P toxin is provided in SEQ ID NO. 6.

Treatment of cells. As mentioned above, *B.t.* or recombinant cells expressing a *B.t.* toxin can be treated to prolong the toxin activity and stabilize the cell. The pesticide microcapsule that is formed comprises the *B.t.* toxin within a cellular structure that has been stabilized and will protect the toxin when the microcapsule is applied to the environment of the target pest. Suitable host cells may include either prokaryotes or eukaryotes, normally being limited to those cells which do not produce substances toxic to higher organisms, such

as mammals. However, organisms which produce substances toxic to higher organisms could be used, where the toxic substances are unstable or the level of application sufficiently low as to avoid any possibility of toxicity to a mammalian host. As hosts, of particular interest will be the prokaryotes and the lower eukaryotes, such as fungi.

5 The cell will usually be intact and be substantially in the proliferative form when treated, rather than in a spore form, although in some instances spores may be employed.

Treatment of the microbial cell, e.g., a microbe containing the *B.t.* toxin gene, can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the toxin, nor diminish the cellular capability of protecting the toxin. Examples of chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under mild conditions and for sufficient time to achieve the desired results. Other suitable techniques include treatment with aldehydes, such as glutaraldehyde; anti-infectives, such as zephiran chloride and cetylpyridinium chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Lugol iodine, Bouin's fixative, various acids, and Helly's fixative (See: Humason, 1967); or a combination of physical (heat) and chemical agents that preserve and prolong the activity of the toxin produced in the cell when the cell is administered to the host animal. Examples of physical means are short wavelength radiation such as gamma-radiation and X-radiation, freezing, UV irradiation, lyophilization, and the like. Methods for treatment of microbial cells are disclosed in United States Patent Nos. 10 4,695,455 and 4,695,462, which are incorporated herein by reference.

The cells generally will have enhanced structural stability which will enhance resistance to environmental conditions. Where the pesticide is in a proform, the method of cell treatment should be selected so as not to inhibit processing of the proform to the mature form of the pesticide by the target pest pathogen. For example, formaldehyde will crosslink proteins and could inhibit processing of the proform of a polypeptide pesticide. The method of cell treatment retains at least a substantial portion of the bio-availability or bioactivity of the toxin.

Characteristics of particular interest in selecting a host cell for purposes of production include ease of introducing the *B.t.* gene into the host, availability of expression systems, efficiency of expression, stability of the pesticide in the host, and the presence of auxiliary genetic capabilities. Characteristics of interest for use as a pesticide microcapsule include protective qualities for the pesticide, such as thick cell walls, pigmentation, and intracellular packaging or formation of inclusion bodies; survival in aqueous environments; lack of 30

mammalian toxicity; attractiveness to pests for ingestion; ease of killing and fixing without damage to the toxin; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

Growth of cells. The cellular host containing the *B.t.* insecticidal gene may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage, providing for a selective medium so that substantially all or all of the cells retain the *B.t.* gene. These cells may then be harvested in accordance with conventional ways. Alternatively, the cells can be treated prior to harvesting.

The *B.t.* cells of the invention can be cultured using standard art media and fermentation techniques. Upon completion of the fermentation cycle the bacteria can be harvested by first separating the *B.t.* spores and crystals from the fermentation broth by means well known in the art. The recovered *B.t.* spores and crystals can be formulated into a wettable powder, liquid concentrate, granules or other formulations by the addition of surfactants, dispersants, inert carriers, and other components to facilitate handling and application for particular target pests. These formulations and application procedures are all well known in the art and are used with commercial strains of *B. thuringiensis* (HD-1) active against Lepidoptera, e.g., caterpillars. The *B.t.* isolates (spores and crystals) of the subject invention can be used to control nematode pests.

The *B.t.* toxins of the invention can be administered orally in a unit dosage form such as a capsule, bolus or tablet, or as a liquid drench when used as an anthelmintic in mammals. The drench is normally a solution, suspension or dispersion of the active ingredient, usually in water, together with a suspending agent such as bentonite and a wetting agent or like excipient. Generally, the drenches also contain an antifoaming agent. Drench formulations generally contain from about 0.001 to 0.5% by weight of the active compound. Preferred drench formulations may contain from 0.01 to 0.1% by weight, the capsules and boluses comprise the active ingredient admixed with a carrier vehicle such as starch, talc, magnesium stearate, or dicalcium phosphate.

Where it is desired to administer the toxin compounds in a dry, solid unit dosage form, capsules, boluses or tablets containing the desired amount of active compound usually are employed. These dosage forms are prepared by intimately and uniformly mixing the active ingredient with suitable finely divided diluents, fillers, disintegrating agents and/or binders such as starch, lactose, talc, magnesium stearate, vegetable gums and the like. Such unit dosage formulations may be varied widely with respect to their total weight and content of the

nematode-active agent, depending upon the factors such as the type of host animal to be treated, the severity and type of infection and the weight of the host.

When the active compound is to be administered via an animal feedstuff, it is intimately dispersed in the feed or used as a top dressing or in the form of pellets which may then be added to the finished feed or, optionally, fed separately. Alternatively, the compounds may be administered to animals parenterally, for example, by intraluminal, intramuscular, intratracheal, or subcutaneous injection, in which event the active ingredient is dissolved or dispersed in a liquid carrier vehicle. For parenteral administration, the active material is suitably admixed with an acceptable vehicle, preferably of the vegetable oil variety, such as peanut oil, cotton seed oil and the like. Other parenteral vehicles, such as organic preparations using solketal, glycerol, formal and aqueous parenteral formulations, are also used. The active compound or compounds are dissolved or suspended in the parenteral formulation for administration; such formulations generally contain from 0.005 to 5% by weight of the active compound.

When the toxins are administered as a component of the feed of the animals, or dissolved or suspended in the drinking water, compositions are provided in which the active compound or compounds are intimately dispersed in an inert carrier or diluent. By inert carrier is meant one that will not react with the nematode-active agent and one that may be administered safely to animals. Preferably, a carrier for feed administration is one that is, or may be, an ingredient of the animal ration.

Suitable compositions include feed premixes or supplements in which the active ingredient is present in relatively large amounts and which are suitable for direct feeding to the animal or for addition to the feed either directly or after an intermediate dilution or blending step. Typical carriers or diluents suitable for such compositions include, for example, distillers' dried grains, corn meal, citrus meal, fermentation residues, ground oyster shells, wheat shorts, molasses solubles, corn cob meal, edible bean mill feed, soya grits, crushed limestone and the like.

In addition to having anthelmintic activity within the digestive tract of mammals, spores from nematocidal *B.t.* isolates will pass through the animals' digestive tract, germinate and multiply in the feces, and thereby provide additional control of nematode larva which hatch and multiply therein.

As would be appreciated by a person skilled in the art, the pesticidal concentration will vary widely depending upon the nature of the particular formulation, particularly whether it is a concentrate or to be used directly. The pesticide will be present in at least 1% by weight

and may be 100% by weight. The dry formulations will have from about 1-95% by weight of the pesticide while the liquid formulations will generally be from about 1-60% by weight of the solids in the liquid phase. The formulations will generally have from about 10^2 to about 10^4 cells/mg. These formulations will be administered at about 50 mg (liquid or dry) to 1 kg or more per hectare.

The formulations can be applied to the environment of the nematode pests, e.g., plants, soil, or water by spraying, dusting, sprinkling, or the like.

Mutants. Mutants of the *B.t.* isolates of the subject invention can be made by procedures well known in the art. For example, an asporogenous mutant can be obtained through ethylmethane sulfonate (EMS) mutagenesis. The mutants can be made using ultraviolet light and nitrosoguanidine by procedures well known in the art.

A smaller percentage of the asporogenous mutants will remain intact and not lyse for extended fermentation periods; these strains are designated lysis minus (-). Lysis minus strains can be identified by screening asporogenous mutants in shake flask media and selecting those mutants that are still intact and contain toxin crystals at the end of the fermentation. Lysis minus strains are suitable for a cell treatment process that will yield a protected, encapsulated toxin protein.

To prepare a phage resistant variant of said asporogenous mutant, an aliquot of the phage lysate is spread onto nutrient agar and allowed to dry. An aliquot of the phage sensitive bacterial strain is then plated directly over the dried lysate and allowed to dry. The plates are incubated at 30°C. The plates are incubated for 2 days and, at that time, numerous colonies could be seen growing on the agar. Some of these colonies are picked and subcultured onto nutrient agar plates. These apparent resistant cultures are tested for resistance by cross streaking with the phage lysate. A line of the phage lysate is streaked on the plate and allowed to dry. The presumptive resistant cultures are then streaked across the phage line. Resistant bacterial cultures show no lysis anywhere in the streak across the phage line after overnight incubation at 30°C. The resistance to phage is then reconfirmed by plating a lawn of the resistant culture onto a nutrient agar plate. The sensitive strain is also plated in the same manner to serve as the positive control. After drying, a drop of the phage lysate is plated in the center of the plate and allowed to dry. Resistant cultures showed no lysis in the area where the phage lysate has been placed after incubation at 30°C for 24 hours.

Following are examples which illustrate procedures, including the best mode, for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

5 Example 1 – Culturing *B.t.* Strains

A subculture of a *B.t.* strain can be used to inoculate the following medium, a peptone, glucose, salts medium.

	Bacto Peptone	7.5 g/l
	Glucose	1.0 g/l
10	KH ₂ PO ₄	3.4 g/l
	K ₂ HPO ₄	4.35 g/l
	Salt Solution	5.0 ml/l
	CaCl ₂ Solution	5.0 ml/l
	Salts Solution (100 ml)	
15	MgSO ₄ ·7H ₂ O	2.46 g
	MnSO ₄ ·H ₂ O	0.04 g
	ZnSO ₄ ·7H ₂ O	0.28 g
	FeSO ₄ ·7H ₂ O	0.40 g
	CaCl ₂ Solution (100 ml)	
20	CaCl ₂ ·2H ₂ O	3.66 g
	pH 7.2	

The salts solution and CaCl₂ solution are filter-sterilized and added to the autoclaved and cooked broth at the time of inoculation. Flasks are incubated at 30°C on a rotary shaker at 200 rpm for 64 hr.

25 The above procedure can be readily scaled up to large fermentors by procedures well known in the art.

The *B.t.* spores and crystals, obtained in the above fermentation, can be isolated by procedures well known in the art. A frequently-used procedure is to subject the harvested fermentation broth to separation techniques, e.g., centrifugation.

30

Example 2 – Activity of *Bacillus thuringiensis* Isolates Against *Panagrellus redivivus*

Worms were collected in a tube and allowed to settle for about 15 minutes. The water was decanted and replaced with fresh water three or four times until the water remained clear. 250 µl rinsed nematodes (20-30 worms), and 100 µl of a spore/crystal suspension were added

to 650 μ l water in each well of a multi-well tray. Nematodes were counted and the numbers recorded. After four days, the live worms were counted and percent mortality calculated.

Table 3. Bioassay results

5	U.S. Patent No. 4,948,734	Mortality
	<i>B.t.</i> strain No.	
	PS17	90%
	PS33F2	30%
	PS52A1	100%
10	PS63B	92%
	PS69D1	100%
	Novel <i>B.t.</i> strain No.	
	PS80JJ1	99%
	PS158D5	99%
15	PS167P	96%
	PS169E	100%
	PS177F1	96%
	PS177G	100%
	PS204G4	100%
20	PS204G6	100%
	Control	0%

Tables 4 and 5 show the molecular mass of the alkali-soluble proteins in each novel nematode-active strain, as compared to previously known *B.t.* strains.

Table 4. Previously known nematode-active strains

	<i>B.t.</i> Strain	Approximate Molecular Mass of Proteins (kDa)
	PS17	155, 145, 135
30	PS33F2	140, 94, 86, 68, 65, 62
	PS52A1	57, 45
	PS63B	84, 82, 78
	PS69D1	135, 46, 32

Table 5. New Nematode-Active Strains

Novel <i>B.t.</i> Strain	Approximate Molecular Mass of Proteins (kDa)
PS80JJ1	130, 90, 47, 37
PS158D5	80
PS167P	120
PS169E	150, 128, 33
PS177F1	140, 116, 103, 62
PS177G	135, 125, 107, 98, 62
PS204G4	105, 98, 90, 60, 44, 37
PS204G6	23, 21

Example 3 — Cloning and Expression of a of Novel Toxin Gene from *Bacillus thuringiensis* Strain PS80JJ1

Total cellular DNA was prepared from *Bacillus thuringiensis* (*B.t.*) cells grown to an optical density, at 600 nm, of 1.0. Cells were pelleted by centrifugation and resuspended in protoplast buffer (20 mg/ml lysozyme in 0.3M sucrose, 25mM Tris-Cl (pH 8.0), 25mM EDTA). After incubation at 37°C for 1 hour, protoplasts were lysed by two cycles of freezing and thawing. Nine volumes of a solution of 0.1 M NaCl, 0.1% SDS, 0.1 M Tris-Cl were added to complete lysis. The cleared lysate was extracted twice with phenol:chloroform (1:1). Nucleic acids were precipitated with two volumes of ethanol and pelleted by centrifugation. The pellet was resuspended in TE buffer and RNase was added to a final concentration of 50 µg/ml. After incubation at 37°C for 1 hour, the solution was extracted once each with phenol:chloroform (1:1) and TE-saturated chloroform. DNA was precipitated from the aqueous phase by the addition of one-tenth volume of 3M NaOAc and two volumes of ethanol. DNA was pelleted by centrifugation, washed with 70% ethanol, dried, and resuspended in TE buffer.

An approximately 700-800 bp DNA fragment from a novel PS80JJ1 130 kDa toxin gene was obtained by polymerase chain reaction (PCR) amplification using PS80JJ1 cellular DNA and the following primers:

"Forward": 5' GGACCAGGATTACAGG(TA)GG(AG)(AG)A 3'

(SEQ ID NO. 1)

"Reverse": 5' TAACGTGTAT(AT)CG(CG)TTTTAATTT(TA)GA(CT)TC 3'

(SEQ ID NO. 2).

The DNA fragment was cloned into pBluescript S/K (Stratagene, LaJolla, CA) and partially sequenced by dideoxynucleotide DNA sequencing methodology (Sanger *et al.*, 1977) using Sequenase (US Biochemicals, Cleveland, OH). DNA sequences unique to at least one PS80JJ1 toxin gene were identified by computer comparison with other known δ -endotoxin genes.

The 700-800 bp DNA fragment was radiolabelled with ^{32}P and used in standard hybridizations of Southern blots of PS80JJ1 total cellular DNA. Hybridizing bands included an approximately 1.8 kbp *EcoRI* fragment and an approximately 9.5 kbp *HindIII* fragment. These hybridizing DNA bands contain toxin genes or restriction fragments of toxin genes from PS80JJ1.

A gene library was constructed from PS80JJ1 DNA partially digested with *NdeII*. Partial restriction digests were fractionated by agarose gel electrophoresis. DNA fragments 9.3 to 23 kbp in size were excised from the gel, electroeluted from the gel slice, purified on an Elutip-D ion exchange column (Schleicher and Schuell, Keene, NH), and recovered by ethanol precipitation. The *NdeII* inserts were ligated into *BamHI*-digested LambdaGem-11 (Promega, Madison, WI). Recombinant phage were packaged and plated on *E. coli* KW251 cells. Plaques were screened by hybridization with the probe described above. Hybridizing phage were plaque-purified and used to infect liquid cultures of *E. coli* KW251 cells for isolation of DNA by standard procedures (Maniatis *et al.*).

For subcloning the gene encoding the PS80JJ1 130 kDa toxin, preparative amounts of phage DNA were digested with *XhoI* and electrophoresed on an agarose gel. The approximately 12 kbp band containing the toxin gene was excised from the gel, electroeluted from the gel slice, and purified by ion exchange chromatography as described above. The purified DNA insert was ligated into *XhoI*-digested pHTBlueII (an *E. coli/B. thuringiensis* shuttle vector comprised of pBluescript S/K [Stratagene, La Jolla, CA] and the replication origin from a resident *B.t.* plasmid [Lereclus *et al.*]). The ligation mix was used to transform frozen, competent *E. coli* NM522 cells (ATCC 47000). β -galactosidase- transformants were screened by restriction digestion of alkaline lysate plasmid minipreps as above. The desired plasmid construct, pMYC2379, contains a toxin gene that is novel compared to other toxin genes containing insecticidal proteins.

The PS80JJ1 toxin gene encoded by pMYC2379 was sequenced using the ABI373 automated sequencing system and associated software. Sequence analysis of the toxin gene

revealed that it encodes a protein of approximately 130,000 daltons, deduced from the DNA sequence. The nucleotide and deduced amino acid sequences are shown in SEQ ID NOS. 3 and 4, respectively.

pMYC2379 was introduced into the acrystalliferous (Cry⁻) *B.t.* host, CryB (A. Aronson, Purdue University, West Lafayette, IN), by electroporation. Expression of the 130kDa toxin was demonstrated by SDS-PAGE analysis.

A subculture of *E. coli* NM522 containing plasmid pMYC2379 was deposited in the permanent collection of the Patent Culture Collection (NRRL), Regional Research Center, 1815 North University Street, Peoria, Illinois 61604 USA on November 3, 1991. The accession number is NRRL B-21155.

Example 4 -- Cloning and Expression of a Novel Toxin Gene from *Bacillus thuringiensis* PS167P

Total cellular DNA was prepared as in Example 3.

An approximately 700-800 bp DNA fragment from novel PS167P 130 kDa toxin genes was obtained by polymerase chain reaction (PCR) amplification using PS167P cellular DNA and SEQ ID NOS. 1 and 2. This DNA fragment was cloned into pBluescript S/K (Stratagene, La Jolla, CA) and partially sequenced by dideoxynucleotide DNA sequencing methodology (Sanger *et al.*, 1977) using Sequenase (U.S. Biochemicals, Cleveland, OH). DNA sequences unique to at least two PS167P toxin genes were identified by computer comparison with other known δ -endotoxin genes.

The 700-800 bp DNA fragment was radiolabelled with ³²P and used in standard hybridizations of Southern blots of PS167P total cellular DNA. Hybridizing bands included approximately 1.8 kbp and 2.3 kbp *EcoRI* fragments and approximately 5.5 kbp and 8.0 kbp *HindIII* fragments. These DNA fragments contain toxin genes or restriction fragments of toxin genes unique to PS167P.

A gene library was constructed from PS167P DNA partially digested with *NdeII*. Partial restriction digests were fractionated by agarose gel electrophoresis. DNA fragments 9.3 to 23 kbp in size were excised from gel, electroeluted from the gel slice, purified on an Elutip-D ion exchange column (Schleicher and Schuell, Keene, NH), and recovered by ethanol precipitation. The *NdeII* inserts were ligated into *BamHI*-digested LambdaGem-11 (Promega, Madison, WI). Recombinant phage were packaged and plated on *E. coli* KW251 cells. Plaques were screened by hybridization with the probe described above. Hybridizing phage

were plaque-purified and used to infect liquid cultures of *E. coli* KW251 cells for isolation of DNA by standard procedures (Maniatis *et al.*, 1989).

Southern blot analysis revealed that one of the recombinant phage isolates contained an approximately 5 kbp *Sa*I band that hybridized to the PS167P toxin gene probe. One of the *Sa*I sites flanking the PS167P toxin gene resides in the phage vector DNA sequence, while the other flanking *Sa*I site is located within the PS167P DNA insert. This *Sa*I fragment was subcloned by standard methods into pBluescript S/K (Stratagene, San Diego, CA) for DNA sequence analysis. The DNA insert was subcloned further as a *Sac*I-*Kpn*I fragment into pHTBlueII (an *E. coli*/*B. thuringiensis* shuttle vector comprised of pBluescript S/K and the replication origin from a resident *B.t.* plasmid [Lereclus *et al.*, 1989] to yield pMYC2382. To test expression of the PS167P toxin gene in *B.t.*, pMYC2382 was introduced into the acrycstalliferous (Cry-) *B.t.* host, CryB (A. Aronson, Purdue University, West Lafayette, IN) by electroporation. Expression of the approximately 130 kDa PS167P toxin encoded by pMYC2382 was demonstrated by SDS-PAGE analysis.

The PS167P toxin gene encoded by pMYC2382 was sequenced using the ABI373 automated sequencing system and associated software. The PS167P toxin nucleotide (SEQ ID NO. 5) and deduced amino acid (SEQ ID NO. 6) sequences are novel compared to other toxin genes encoding pesticidal proteins.

A subculture of *E. coli* NM522 containing plasmid pMYC2382 was deposited in the permanent collection of the Patent Culture Collection (NRRL), Regional Research Center, 1815 North University Street, Peoria, Illinois 61604 USA on September 28, 1994. The accession number is NRRL B-21329.

Example 5 — Insertion of Toxin Genes Into Plants

One aspect of the subject invention is the transformation of plants with genes encoding a toxin active against nematode pests. The transformed plants are resistant to attack by nematodes.

Genes encoding pesticidal toxins, as disclosed herein, can be modified for optimum expression in plant, linked to a plant selectable marker gene, and inserted into a genome of plant cell using a variety of techniques which are well known to those skilled in the art. Any plant may be used in accordance with this invention, including angiosperms, gymnosperms, monocotyledons and dicotyledons. Preferred plants include soybean, sunflower, cotton, potato, alfalfa, maize, rice and wheat. The transformation method itself is not critical to the invention but may include transformation with T-DNA using *Agrobacterium tumefaciens* or

A. rhizogenes as the transformation agent, liposome fusion, microinjection, microprojectile bombardment, chemical agent (PEG or calcium chloride)-assisted DNA uptake, or electroporation, as well as other possible methods. Reference may be made to the literature for full details of the known methods, especially Holsters *et al.*, 1978; Fromm *et al.*, 1985; Horsch *et al.*, 1985; Herrera-Estrella *et al.*, 1983; Crossway *et al.*, 1986; Lin, 1966; and Steinkiss and Stabel, 1983.

Use of a plant selectable marker in transformation allows for selection of transformed cells rather than cells that do not contain the inserted DNA. Various markers exist for use in plant cells and generally provide resistance to a biocide or antibiotic, including but not limited to, kanamycin, G418, hygromycin, and phosphinothricin. Visual markers including but not limited to b-glucuronidase, b-galactosidase, B-peru protein, green fluorescent protein, and luciferase may also be used. After transformation, those cells that have the DNA insert can be selected for by growth in a defined medium and assayed for marker expression, whether by resistance or visualization. Cells containing the DNA insert can be regenerated into plants. As long as stably transformed plants are obtained, the method used for regeneration will depend on the plant tissue and transformation method used and is not critical to the invention. However, for example, where cell suspensions have been used for transformation, transformed cells can be induced to produce calli and the calli subsequently induced to form shoots, which may then be transferred to an appropriate nutrient medium to regenerate plants. Alternatively, explants such as hypocotyl tissue or embryos may be transformed and regenerated by shoot induction in the appropriate media, followed by root and whole plant formation. Whatever regeneration method is used, the result will be stably transformed plants that can vegetatively and sexually transmit the transformed trait(s) to progeny, so that, if necessary, the transformed plant can be crossed with untransformed plants in order to transfer the trait to more appropriate germplasm for breeding purposes.

Example 6 – Cloning of Novel *B.t.* Genes Into Insect Viruses

A number of viruses are known to infect insects. These viruses include, for example, baculoviruses and entomopoxviruses. In one embodiment of the subject invention, nematode-active genes, as described herein, can be placed with the genome of the insect virus, thus enhancing the pathogenicity of the virus. Methods for constructing insect viruses which comprise *B.t.* toxin genes are well known and readily practiced by those skilled in the art. These procedures are described, for example, in Merryweather *et al.* (1990) and Martens *et al.* (1990).

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

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U.S. Patent No. 4,467,036.
U.S. Patent No. 4,695,455
U.S. Patent No. 4,695,462.
U.S. Patent No. 4,797,276.
U.S. Patent No. 4,849,217.
U.S. Patent No. 4,853,331.
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(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(ix) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/918,345
(B) FILING DATE: 21-JUL-1992
(C) CLASSIFICATION:

(x) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/558,738
(B) FILING DATE: 27-JUL-1990

25

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

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- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: US 08/176,403
- (B) FILING DATE: 30-DEC-1993
- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGACCAGGAT TTACAGGWGG RRA

23

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TAACGTGTAT WCGSTTTTAA TTIWGAYTC

29

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3561 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGGATTGTA ATTTACAATC ACAACAAAAT ATTCCTTATA ATGTATTAGC AATACCAGTA	60
TCTAATGTTA ATGCGTTGGT TGATACAGCT GGAGATTAA AAAAGCATG GGAAGAATTT	120
CAAAAACTG GTTCTTTTTC ATTAACAGCT TTACAACAAG GATTTTCTGC CTCACAAGGA	180
GGAGCATTCA ATTATTTAA CATTATTACA TCAGGAATAT CATTAGCTGG TTCCTTTGTC	240
CCTGGAGGTA CTTTGTAGC ACCCATTGTT AATATGGTTA TTGGTTGGTT ATGCCACAT	300
AAAAACAAGA CAGCGGATAC AGAAAAATTA ATAAATTAA TTGATGAAGA AATTCAAAAA	360
CAATTAAACA AAGCCTTATT AGACCAAGAT AGAAACAATT GGACCTCTTT TTTAGAAAGT	420
ATATTGATA CTTCAGCTAC AGTAAGTAAT GCAATTATAG ATGCACAGTG GTCAGGTACT	480
GTAGATACTA CAAATAGACA ACAAAAAACT CCAACAACAT CAGATTATCT AAATGTTGTT	540
GGAAAATTTG ATTCAGCGGA TTCTTCAATT ATAACAAATG AAAATCAAAT AATGAATGGC	600
AACTTTGACG TAGCTGCAGC ACCCTATTTT GTTATAGGAG CAACATTACG TCTTTCATTA	660
TATCAATCTT ATATTAAAT TTGTAATAGT TGGATTGATG CAGTTGGATT TAGTACAAAT	720
GATGCTAATA CACAAAAAGC TAATTTAGCT CGTACGAAAT TAACTATGCG TACTACAATT	780
AATGAATATA CACAAGAGT TATGAAAGTT TTTAAAGATT CCAAGAATAT GCCTACAATA	840
GGTACTAATA AATTTAGTGT TGATGCTTAT AATGTATATG TTAAGGAAT GACATTAAAT	900
GTTTTAGATA TGGTAGCAAT ATGGTCTTCA TTATATCCAA ATGATTATAC TTCACAAACA	960
GCCATAGAAC AAACACGTGT CACTTTTTC AATATGGTTG GACAGAAGA AGGTACAGAT	1020
GGAAACCTAA AAATTACAA TACTTTTGAT TCTCTTAGTT ATCAACATAG CCTAATACCT	1080
AATAATAATG TTAATTTAAT TTCTTATTAT ACTGATGAAT TGCAAAATCT AGAATTAGCA	1140
GTATATACTC CTAAGGTGG AAGTGGATAC GCTTATCCTT ATGGATTTAT TTTAAATTAT	1200
GCAACACGCA ACTACAAATA TGGTGATAAT GATCCAACAG GCAACACATT AAATAACAAC	1260

GATGGACCTA	TACAACAAAT	AAATGCAGCA	ACTCAAAACA	GTAAATATCT	AGATGGAGAA	1320
ACAATAAATG	GAATAGGGGC	ATCCTTACCT	GGTTATTGTA	CTACAGGATG	TTCAGCAACA	1380
GAACAACCTT	TTAGTTGTAC	TTCTACTGCT	AATAGCTATA	AAGCAAGCTG	TAATCCTTCA	1440
GATACTAATC	AAAAAATTAA	TGCTTTATAT	GCTTTTACAC	AAACTAATGT	AAAGGGAAGC	1500
ACGGGGAAT	TAGGAGTACT	GGCAAGTCTT	GTCCATATG	ATTTAAATCC	TAAAAATGTA	1560
TTTGGTGAAT	TAGATTGAGA	TACAAATAAT	GTTATCTTAA	AAGGAATTC	TGCAGAAAAA	1620
GGGTATTTTC	CTAATAATGC	GCGACCTACT	GTGTAAAG	AATGGATTAA	TGTTGCAAGT	1680
GCTGTACCAT	TTTATTCAGG	AAATACTTTA	TTTATGACGG	CTACGAATT	AACAGCTACT	1740
CAATATAAAA	TTAGAATACG	TTATGCAAA	CCAAATTCAG	ATACTCAAA	CGGTGTACTA	1800
ATTACGCAA	ATGGTTCTCA	AATTTCCAAT	AGTAATCTAA	CACTTTATAG	TACTACTGAT	1860
TCAAGTATGA	GTAGTAATTT	ACCACAAAAT	GTATATGTCA	CAGGGGAAAA	TGGAAATTAT	1920
ACACTTCTAG	ATTTATATAG	TACTACTAAT	GTTTTATCAA	CAGGAGATAT	TACATTAAAA	1980
CTTACAGGAG	GAAATCAAAA	AATATTATT	GATCGAATAG	AATTTATCC	TACTATGCCT	2040
GTACCTGCTC	CTACTAATA	CACTAATAAC	AATAACGGCG	ATAACGGCAA	TAACATCCC	2100
CCACACCACG	GTTGTGCAAT	AGCTGGTACA	CAACAACCTT	GTTCTGGACC	ACCTAAGTTT	2160
GAACAAGTAA	GTGATTTAGA	AAAAATTACA	ACGCAAGTAT	ATATGTTATT	CAATCTTCT	2220
TCGTATGAAG	AATTAGCTCT	AAAAGTTTCT	AGCTATCAA	TTAATCAAGT	GGCATTGAAA	2280
GTTATGGCAC	TATCTGATGA	AAAGTTTTGT	GAAGAAAAAA	GATTGTTACG	AAAATTAGTC	2340
AATAAAGCAA	ACCAATTACT	AGAAGCACGT	AACTTACTAG	TAGTGGGAAA	TTTTGAAACA	2400
ACTCAAAATT	GGGTACTTGG	AACAAATGCT	TATATAAATT	ATGATTGCTT	TTTATTTAAT	2460
GGAAATTATT	TATCCTTACA	ACCAGCAAGT	GGATTTTICA	CATCTTATGC	TTATCAAAAA	2520
ATAGATGAGT	CAACATTAAA	ACCATATACA	CGATATAAAG	TTTCTGGATT	CATTGGGCAA	2580
AGTAATCAAG	TAGAACTTAT	TATTTCTCGT	TATGGAAAAG	AAATTGATAA	AATATTAAAT	2640
GTTCATATG	CAGGCGCTCT	TCCTATTACT	GCTGATGCAT	CGATAACTTG	TTGTGCACCA	2700
GAAATAGACC	AATGTGATGG	GGGGCAATCT	GATTCTCATT	TCTTCAACTA	TAGCATCGAT	2760
GTAGGTGCAC	TTCACCCAGA	ATTAAACCCT	GGCATTGAAA	TTGTCCTTAA	AATTGTGCAA	2820
TCAAATGGTT	ATATAACAAT	TAGTAATCTA	GAAATTATTG	AAGAAGCTCC	ACTTACAGAA	2880
ATGGAAATTC	AAGCAGTCAA	TCGAAAAGAT	CACAAATGGA	AAAGAGAAAA	ACTTCTAGAA	2940

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TGTGCAAGTG TTAGTGAACT TTTACAACCA ATCATTAAATC AAATCGATTG ATTGTTCAAA      3000
GATGCAAACT GGTATAATGA TATTCTTCCT CATGTCACAT ATCAAATCTT AAAAAATATT      3060
ATAGTACCCG ATTTACCAAA ATTAATAACAT TGGTTCATAG ATCATCTCCC AGGTGAATAT      3120
CATGAAATTG AACAAACAAT GAAAGAAGCT CTAAACATG CATTACACA ATTAGACGAG      3180
AAAAATTTAA TCCACAATGG TCACTTTGCA ACTAACTTAA TAGATTGGCA AGTAGAAGGT      3240
GATGCTCGAA TGAAAGTATT AGAAAAATAAT GCTTTGGCAT TACAACTTTC CAATTGGGAT      3300
TCTAGTGTTT CACAATCTAT TGATATATTA GAATTTGATG AAGATAAAGC ATATAAACTT      3360
CGCGTATATG CTCAAGGAAG CGGAACAATC CAATTGGGAA ACTGTGAAGA TGAAGCCATC      3420
CAATTTAATA CAAACTCATT CGTATATAAA GAAAAAATAA TCTATTTCGA TACCCCATCA      3480
ATTAACCTAC ACATACAATC AGAAGGTTCT GAATTCGTTG TAAGTAGTAT CGACCTCGTT      3540
GAATTATCAG ACGACGAATA A                                     3561

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1186 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Asp Cys Asn Leu Gln Ser Gln Gln Asn Ile Pro Tyr Asn Val Leu
1             5             10             15

Ala Ile Pro Val Ser Asn Val Asn Ala Leu Val Asp Thr Ala Gly Asp
20             25             30

Leu Lys Lys Ala Trp Glu Glu Phe Gln Lys Thr Gly Ser Phe Ser Leu
35             40             45

Thr Ala Leu Gln Gln Gly Phe Ser Ala Ser Gln Gly Gly Ala Phe Asn
50             55             60

Tyr Leu Thr Leu Leu Gln Ser Gly Ile Ser Leu Ala Gly Ser Phe Val
65             70             75             80

Pro Gly Gly Thr Phe Val Ala Pro Ile Val Asn Met Val Ile Gly Trp
85             90             95

Leu Trp Pro His Lys Asn Lys Thr Ala Asp Thr Glu Asn Leu Ile Lys
100            105            110

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Leu Ile Asp Glu Glu Ile Gln Lys Gln Leu Asn Lys Ala Leu Leu Asp
 115 120 125
 Gln Asp Arg Asn Asn Trp Thr Ser Phe Leu Glu Ser Ile Phe Asp Thr
 130 135 140
 Ser Ala Thr Val Ser Asn Ala Ile Ile Asp Ala Gln Trp Ser Gly Thr
 145 150 155 160
 Val Asp Thr Thr Asn Arg Gln Gln Lys Thr Pro Thr Thr Ser Asp Tyr
 165 170 175
 Leu Asn Val Val Gly Lys Phe Asp Ser Ala Asp Ser Ser Ile Ile Thr
 180 185
 Asn Glu Asn Gln Ile Met Asn Gly Asn Phe Asp Val Ala Ala Ala Pro
 195 200 205
 Tyr Phe Val Ile Gly Ala Thr Leu Arg Leu Ser Leu Tyr Gln Ser Tyr
 210 215 220
 Ile Lys Phe Cys Asn Ser Trp Ile Asp Ala Val Gly Phe Ser Thr Asn
 225 230 235 240
 Asp Ala Asn Thr Gln Lys Ala Asn Leu Ala Arg Thr Lys Leu Thr Met
 245 250 255
 Arg Thr Thr Ile Asn Glu Tyr Thr Gln Arg Val Met Lys Val Phe Lys
 260 265 270
 Asp Ser Lys Asn Met Pro Thr Ile Gly Thr Asn Lys Phe Ser Val Asp
 275 280 285
 Ala Tyr Asn Val Tyr Val Lys Gly Met Thr Leu Asn Val Leu Asp Met
 290 295 300
 Val Ala Ile Trp Ser Ser Leu Tyr Pro Asn Asp Tyr Thr Ser Gln Thr
 305 310 315 320
 Ala Ile Glu Gln Thr Arg Val Thr Phe Ser Asn Met Val Gly Gln Glu
 325 330 335
 Glu Gly Thr Asp Gly Thr Leu Lys Ile Tyr Asn Thr Phe Asp Ser Leu
 340 345 350
 Ser Tyr Gln His Ser Leu Ile Pro Asn Asn Asn Val Asn Leu Ile Ser
 355 360 365
 Tyr Tyr Thr Asp Glu Leu Gln Asn Leu Glu Leu Ala Val Tyr Thr Pro
 370 375 380
 Lys Gly Gly Ser Gly Tyr Ala Tyr Pro Tyr Gly Phe Ile Leu Asn Tyr
 385 390 395 400

Ala Asn Ser Asn Tyr Lys Tyr Gly Asp Asn Asp Pro Thr Gly Lys Pro
 405 410 415
 Leu Asn Lys Gln Asp Gly Pro Ile Gln Gln Ile Asn Ala Ala Thr Gln
 420 425 430
 Asn Ser Lys Tyr Leu Asp Gly Glu Thr Ile Asn Gly Ile Gly Ala Ser
 435 440 445
 Leu Pro Gly Tyr Cys Thr Thr Gly Cys Ser Ala Thr Glu Gln Pro Phe
 450 455 460
 Ser Cys Thr Ser Thr Ala Asn Ser Tyr Lys Ala Ser Cys Asn Pro Ser
 465 470 475 480
 Asp Thr Asn Gln Lys Ile Asn Ala Leu Tyr Ala Phe Thr Gln Thr Asn
 485 490 495
 Val Lys Gly Ser Thr Gly Lys Leu Gly Val Leu Ala Ser Leu Val Pro
 500 505 510
 Tyr Asp Leu Asn Pro Lys Asn Val Phe Gly Glu Leu Asp Ser Asp Thr
 515 520 525
 Asn Asn Val Ile Leu Lys Gly Ile Pro Ala Glu Lys Gly Tyr Phe Pro
 530 535 540
 Asn Asn Ala Arg Pro Thr Val Val Lys Glu Trp Ile Asn Gly Ala Ser
 545 550 555 560
 Ala Val Pro Phe Tyr Ser Gly Asn Thr Leu Phe Met Thr Ala Thr Asn
 565 570 575
 Leu Thr Ala Thr Gln Tyr Lys Ile Arg Ile Arg Tyr Ala Asn Pro Asn
 580 585 590
 Ser Asp Thr Gln Ile Gly Val Leu Ile Thr Gln Asn Gly Ser Gln Ile
 595 600 605
 Ser Asn Ser Asn Leu Thr Leu Tyr Ser Thr Thr Asp Ser Ser Met Ser
 610 615 620
 Ser Asn Leu Pro Gln Asn Val Tyr Val Thr Gly Glu Asn Gly Asn Tyr
 625 630 635 640
 Thr Leu Leu Asp Leu Tyr Ser Thr Thr Asn Val Leu Ser Thr Gly Asp
 645 650 655
 Ile Thr Leu Lys Leu Thr Gly Gly Asn Gln Lys Ile Phe Ile Asp Arg
 660 665 670
 Ile Glu Phe Ile Pro Thr Met Pro Val Pro Ala Pro Thr Asn Asn Thr
 675 680 685

Asn	Asn	Asn	Asn	Gly	Asp	Asn	Gly	Asn	Asn	Asn	Pro	Pro	His	His	Gly
690						695					700				
Cys	Ala	Ile	Ala	Gly	Thr	Gln	Gln	Leu	Cys	Ser	Gly	Pro	Pro	Lys	Phe
705					710					715					720
Glu	Gln	Val	Ser	Asp	Leu	Glu	Lys	Ile	Thr	Thr	Gln	Val	Tyr	Met	Leu
				725					730					735	
Phe	Lys	Ser	Ser	Ser	Tyr	Glu	Glu	Leu	Ala	Leu	Lys	Val	Ser	Ser	Tyr
			740					745					750		
Gln	Ile	Asn	Gln	Val	Ala	Leu	Lys	Val	Met	Ala	Leu	Ser	Asp	Glu	Lys
			755				760						765		
Phe	Cys	Glu	Glu	Lys	Arg	Leu	Leu	Arg	Lys	Leu	Val	Asn	Lys	Ala	Asn
	770					775						780			
Gln	Leu	Leu	Glu	Ala	Arg	Asn	Leu	Leu	Val	Gly	Gly	Asn	Phe	Glu	Thr
785					790					795					800
Thr	Gln	Asn	Trp	Val	Leu	Gly	Thr	Asn	Ala	Tyr	Ile	Asn	Tyr	Asp	Ser
				805					810					815	
Phe	Leu	Phe	Asn	Gly	Asn	Tyr	Leu	Ser	Leu	Gln	Pro	Ala	Ser	Gly	Phe
			820						825					830	
Phe	Thr	Ser	Tyr	Ala	Tyr	Gln	Lys	Ile	Asp	Glu	Ser	Thr	Leu	Lys	Pro
			835				840						845		
Tyr	Thr	Arg	Tyr	Lys	Val	Ser	Gly	Phe	Ile	Gly	Gln	Ser	Asn	Gln	Val
	850					855					860				
Glu	Leu	Ile	Ile	Ser	Arg	Tyr	Gly	Lys	Glu	Ile	Asp	Lys	Ile	Leu	Asn
865					870					875					880
Val	Pro	Tyr	Ala	Gly	Pro	Leu	Pro	Ile	Thr	Ala	Asp	Ala	Ser	Ile	Thr
				885					890					895	
Cys	Cys	Ala	Pro	Glu	Ile	Asp	Gln	Cys	Asp	Gly	Gly	Gln	Ser	Asp	Ser
			900					905						910	
His	Phe	Phe	Asn	Tyr	Ser	Ile	Asp	Val	Gly	Ala	Leu	His	Pro	Glu	Leu
			915				920						925		
Asn	Pro	Gly	Ile	Glu	Ile	Gly	Leu	Lys	Ile	Val	Gln	Ser	Asn	Gly	Tyr
	930					935							940		
Ile	Thr	Ile	Ser	Asn	Leu	Glu	Ile	Ile	Glu	Glu	Arg	Pro	Leu	Thr	Glu
945					950					955					960
Met	Glu	Ile	Gln	Ala	Val	Asn	Arg	Lys	Asp	His	Lys	Trp	Lys	Arg	Glu
				965					970					975	

32

Lys Leu Leu Glu Cys Ala Ser Val Ser Glu Leu Leu Gln Pro Ile Ile
 980 985 990
 Asn Gln Ile Asp Ser Leu Phe Lys Asp Ala Asn Trp Tyr Asn Asp Ile
 995 1000 1005
 Leu Pro His Val Thr Tyr Gln Thr Leu Lys Asn Ile Ile Val Pro Asp
 1010 1015 1020
 Leu Pro Lys Leu Lys His Trp Phe Ile Asp His Leu Pro Gly Glu Tyr
 1025 1030 1035 1040
 His Glu Ile Glu Gln Gln Met Lys Glu Ala Leu Lys His Ala Phe Thr
 1045 1050 1055
 Gln Leu Asp Glu Lys Asn Leu Ile His Asn Gly His Phe Ala Thr Asn
 1060 1065 1070
 Leu Ile Asp Trp Gln Val Glu Gly Asp Ala Arg Met Lys Val Leu Glu
 1075 1080 1085
 Asn Asn Ala Leu Ala Leu Gln Leu Ser Asn Trp Asp Ser Ser Val Ser
 1090 1095 1100
 Gln Ser Ile Asp Ile Leu Glu Phe Asp Glu Asp Lys Ala Tyr Lys Leu
 1105 1110 1115 1120
 Arg Val Tyr Ala Gln Gly Ser Gly Thr Ile Gln Phe Gly Asn Cys Glu
 1125 1130 1135
 Asp Glu Ala Ile Gln Phe Asn Thr Asn Ser Phe Val Tyr Lys Glu Lys
 1140 1145 1150
 Ile Ile Tyr Phe Asp Thr Pro Ser Ile Asn Leu His Ile Gln Ser Glu
 1155 1160 1165
 Gly Ser Glu Phe Val Val Ser Ser Ile Asp Leu Val Glu Leu Ser Asp
 1170 1175 1180
 Asp Glu
 1185

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3504 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGACAAATC CAACTATACT ATATCCTAGT TACCATAATG TATTAGTCA TCCGATTAGA	60
TTAGATCTT TTTTGTATCC ATTGTAGAG ACATTTAAGG ATTTAAAGG GCGTGGGAA	120
GAATTCGGAA AAACGGGATA TATGGACCCC TTAACAAC ACCTTCAAT CGCATGGGAT	180
ACTAGTCAAA ATGGAACAGT GGATTATTTA GCATTAACAA AAGCATCTAT ATCTCTCATA	240
GGTTTAATC CTGCTGCAGA CGCTGTAGTC CCTTTTATTA ATATGTTGT AGACTTTATT	300
TTTCCGAAT TATTTGGAAG AGGTCTCAA CAAAATGCTC AAGCTCAATT TTTGGAACATA	360
ATCATAGAAA AAGTTAAAGA ACTTGTGTAT GAAGATTTTA GAACTTTAC CCTTAATAAT	420
CTACTCAATT ACCTTGATGG TATGCAAACA GCCTTATCAC ATTTCCAAAA CGATGTACAA	480
ATTGCTATTT GTCAAGGAGA ACAACCAGGA CTTATGCTAG ATCAAACACC AACGCTTGT	540
ACTCCTACTA CAGACCATT TATTCTGTA AGAGAATCTT TTAAGATGC TCGAACTACA	600
ATTGAAACAG CTTTACCACA TTTTAAAAAT CCTATGCTAT CCACAAATGA TAACACTCCA	660
GATTTTAATA GCGCACTGT CTTATTAACA TTACCAATGT ATACAACAGG AGCGACTTTA	720
AATCTTATAT TACATCAAGG GTATATTCAA TTCGCAGAAA GATGGAAATC TGTAAATTAT	780
GATGAAAGTT TTATAAATCA AACAAAAGTT GATTGCAAC GTCGTATTCA GGACTATTCT	840
ACTACTGTAT CTACCACTTT TGAATAATTC AACCTACTC TAAATCCATC AAATAAGAA	900
TCTGTTAATA AGTATAATAG ATATGTTCTG TCCATGACTC TTCAATCTTT AGACATTGCT	960
GCAACATGGC CTACTTTAGA TAATGTTAAT TACCCTTCCA ATGTAGATAT TCAATTGGAT	1020
CAAACTCGCT TAGTATTTTC AGATGTTGCA GGACCTTGGG AAGGTAATGA TAATATAACT	1080
TCGAATATTA TAGATGTATT AACACCAATA AATACAGGGA TAGGATTICA AGAAAGTTCA	1140
GATCTTAGAA AATTCACCTA TCCACGAATA GAATTACAAA GCATGCAATT CCATGGACAA	1200
TATGTAAACT CAAAAGTGT AGAACATTGT TATAGCGATG GTCTTAAATT AAATTATAAA	1260
AATAAACTA TAACTGCAGG TGTAAGTAAT ATTGATGAAA GTAATCAAAA TAATAACAT	1320
AACTATGGTC CTGTAATAAA TAGTCCTATT ACTGATATCA ACGTAAATTC CCAAAATTCT	1380
CAATATTTAG ATTTAAATTC AGTCATGGTA AATGGTGGTC AAAAAGTAAC CGGGTGTTC	1440
CCACTTAGTT CAAATGGTAA TTCTAATAAT GCTGCTTTAC CTAATCAAAA AATAAATGTT	1500
ATTTATTCAG TACAATCAAA TGATAAACCA GAAAAACATG CAGACACTTA TAGAAAAATGG	1560
GGATATATGA GCAGTCATAT TCCTTATGAT CTGTGTCAG AAAATGTAAT TGGAGATATA	1620
GATCCGGATA CTAAACAACC GTCATTGCTT CTAAAGGCT TTCCGGCAGA AAAAGGATAT	1680

GGTGACTCAA TTGCATATGT ATCAGAACCT TTAATGGTG CGAATGCAGT TAAACTTACT	1740
TCATATCAAG TTCTCCAAAT GGAAGTTACA AATCAACAA CTCAAAAATA TCGTATTTCG	1800
ATACGTTATG CTACAGGTGG AGATACAGCT GCTTCTATAT GGTTCATAT TATTGGTCCA	1860
TCTGGAAATG ATTTAACAAA CGAAGGCCAT AACTTCTCTA GTGTATCTTC TAGAAATAAA	1920
ATGTTTGTTC AGGGTAATAA CGGAAAATAT GTATTGAACA TCCTTACAGA TTCAATAGAA	1980
TTACCATCAG GACAACAAC TATTCTTATT CAAAATACTA ATTCTCAAGA TCTTTTTTIA	2040
GATCGTATTG AATTTATTTC TCTCCCTTCT ACTTCTACTC CTACTTCTAC TAATTTTGTA	2100
GAACCTGAAT CATTAGAAAA GATCATAAAC CAAGTTAATC AATTATTTAG CTCCTCATCT	2160
CAAACTGAAT TGCTCACAC TGTAAAGCGAT TATAAAATTG ATCAAGTAGT GCTAAAAGTA	2220
AATGCCTTAT CCGACGATGT ATTTGGTGTA GAGAAAAAG CATTACGTAA ACTTGTGAAT	2280
CAGGCCAAAC AACTCAGTAA AGCACGAAAT GTATTGGTCG GTGGAACTT TGA AAAAGGT	2340
CATGAATGGG CACTAAGCCG TGAAGCAACA ATGGTCGCAA ATCATGAGTT ATTCAAAGGG	2400
GATCATTTAT TATTACCACC ACCAACCCCTA TATCCATCGT ATGCATATCA AAAAATTGAT	2460
GAATCGAAT TAAAACCAA TACACGTTAT ACTGTTTCGG GCTTTATTGC GCAAAGTGAA	2520
CATCTAGAAG TCGTTGTGTC TCGATACGGG AAAGAAGTAC ATGACATGTT AGATATCCCG	2580
TATGAAGAAG CCTTACCAAT TTCTTCTGAT GAGAGTCCAA ATTGTTGCAA ACCAGCTGCT	2640
TGTCAGTGT CATCTTGTA TGGTAGTCAA TCAGATTCTC ATTTCTTTAG CTATAGTATC	2700
GATGTTGGTT CCTTACAATC AGATGTAAAT CTCGGCATTG AATTCGGTCT TCGTATTGCG	2760
AAACCAACG GATTTCGAA AATCAGTAAT CTAGAAATTA AAGAAGATCG TCCATTAAACA	2820
GAAAAGAGAA TCAAAAAAGT ACAACGTAAA GAACAAAAAT GGA AAAAAGC ATTTAACCAA	2880
GAACAAGCCG AAGTAGCGAC AACACTCCAA CCAACGTTAG ATCAAATCAA TGCTTTGTAT	2940
CAAAATGAAG ATTGGAACGG TTCCGTTTAC CCGGCCAGTG ACTATCAACA TCTGTCGGCT	3000
GTGTTGTAC CAACGTTACC AAAACAAGA CATTGGTTTA TGGAGGGTGC AGAAGGCGAA	3060
CATGTTGTTC TGACGCAACA ATTCCAACAA GCATTGGATC GTGCGTTCCA ACAAATCGAA	3120
GAACAAAAT TAATCCACAA TGTAAATTTG GCGAATGGAT TAACAGATTG GACTGTCACA	3180
GGAGATGCAC AACTTACGAT CTTTGACGAA GATCCAGTAT TAGAACTAGC GCATTGGGAT	3240
GCAAGTATCT CTCAAACCAT TGAAATTATG GATTTTGAAG GAAGACACAG AATACAAACT	3300
GCGTGTACGT GGA AAAAGGCA AAGGAACAGT TACCGTTCAA CATGGAGGAA GAGATTAGAA	3360

ACGATGACAT TCAATACAAC GAGTTTACAC ACACAAGAAC AAACCTTCTA CTCGAAGGA 3420
 GATACAGTGG ACGTACATGT TCAATCAGAG AATAACACAT TCCTGATAGA TAGTGTGGAA 3480
 CTCATTGAAA TCATAGAAGA GTAA 3504

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1167 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Thr Asn Pro Thr Ile Leu Tyr Pro Ser Tyr His Asn Val Leu Ala
 1 5 10 15
 His Pro Ile Arg Leu Asp Ser Phe Phe Asp Pro Phe Val Glu Thr Phe
 20 25 30
 Lys Asp Leu Lys Gly Ala Trp Glu Glu Phe Gly Lys Thr Gly Tyr Met
 35 40 45
 Asp Pro Leu Lys Gln His Leu Gln Ile Ala Trp Asp Thr Ser Gln Asn
 50 55 60
 Gly Thr Val Asp Tyr Leu Ala Leu Thr Lys Ala Ser Ile Ser Leu Ile
 65 70 75 80
 Gly Leu Ile Pro Gly Ala Asp Ala Val Val Pro Phe Ile Asn Met Phe
 85 90 95
 Val Asp Phe Ile Phe Pro Lys Leu Phe Gly Arg Gly Ser Gln Gln Asn
 100 105 110
 Ala Gln Ala Gln Phe Phe Glu Leu Ile Ile Glu Lys Val Lys Glu Leu
 115 120 125
 Val Asp Glu Asp Phe Arg Asn Phe Thr Leu Asn Asn Leu Leu Asn Tyr
 130 135 140
 Leu Asp Gly Met Gln Thr Ala Leu Ser His Phe Gln Asn Asp Val Gln
 145 150 155 160
 Ile Ala Ile Cys Gln Gly Glu Gln Pro Gly Leu Met Leu Asp Gln Thr
 165 170 175
 Pro Thr Ala Cys Thr Pro Thr Thr Asp His Leu Ile Ser Val Arg Glu
 180 185 190

Ser Phe Lys Asp Ala Arg Thr Thr Ile Glu Thr Ala Leu Pro His Phe
 195 200 205
 Lys Asn Pro Met Leu Ser Thr Asn Asp Asn Thr Pro Asp Phe Asn Ser
 210 215 220
 Asp Thr Val Leu Leu Thr Leu Pro Met Tyr Thr Thr Gly Ala Thr Leu
 225 230 235 240
 Asn Leu Ile Leu His Gln Gly Tyr Ile Gln Phe Ala Glu Arg Trp Lys
 245 250 255
 Ser Val Asn Tyr Asp Glu Ser Phe Ile Asn Gln Thr Lys Val Asp Leu
 260 265 270
 Gln Arg Arg Ile Gln Asp Tyr Ser Thr Thr Val Ser Thr Thr Phe Glu
 275 280 285
 Lys Phe Lys Pro Thr Leu Asn Pro Ser Asn Lys Glu Ser Val Asn Lys
 290 295 300
 Tyr Asn Arg Tyr Val Arg Ser Met Thr Leu Gln Ser Leu Asp Ile Ala
 305 310 315 320
 Ala Thr Trp Pro Thr Leu Asp Asn Val Asn Tyr Pro Ser Asn Val Asp
 325 330 335
 Ile Gln Leu Asp Gln Thr Arg Leu Val Phe Ser Asp Val Ala Gly Pro
 340 345 350
 Trp Glu Gly Asn Asp Asn Ile Thr Ser Asn Ile Ile Asp Val Leu Thr
 355 360 365
 Pro Ile Asn Thr Gly Ile Gly Phe Gln Glu Ser Ser Asp Leu Arg Lys
 370 375 380
 Phe Thr Tyr Pro Arg Ile Glu Leu Gln Ser Met Gln Phe His Gly Gln
 385 390 395 400
 Tyr Val Asn Ser Lys Ser Val Glu His Cys Tyr Ser Asp Gly Leu Lys
 405 410 415
 Leu Asn Tyr Lys Asn Lys Thr Ile Thr Ala Gly Val Ser Asn Ile Asp
 420 425 430
 Glu Ser Asn Gln Asn Asn Lys His Asn Tyr Gly Pro Val Ile Asn Ser
 435 440 445
 Pro Ile Thr Asp Ile Asn Val Asn Ser Gln Asn Ser Gln Tyr Leu Asp
 450 455 460
 Leu Asn Ser Val Met Val Asn Gly Gly Gln Lys Val Thr Gly Cys Ser
 465 470 475 480

Pro Leu Ser Ser Asn Gly Asn Ser Asn Asn Ala Ala Leu Pro Asn Gln
 485 490 495
 Lys Ile Asn Val Ile Tyr Ser Val Gln Ser Asn Asp Lys Pro Glu Lys
 500 505 510
 His Ala Asp Thr Tyr Arg Lys Trp Gly Tyr Met Ser Ser His Ile Pro
 515 520 525
 Tyr Asp Leu Val Pro Glu Asn Val Ile Gly Asp Ile Asp Pro Asp Thr
 530 535 540
 Lys Gln Pro Ser Leu Leu Lys Gly Phe Pro Ala Glu Lys Gly Tyr
 545 550 555 560
 Gly Asp Ser Ile Ala Tyr Val Ser Glu Pro Leu Asn Gly Ala Asn Ala
 565 570 575
 Val Lys Leu Thr Ser Tyr Gln Val Leu Gln Met Glu Val Thr Asn Gln
 580 585 590
 Thr Thr Gln Lys Tyr Arg Ile Arg Ile Arg Tyr Ala Thr Gly Gly Asp
 595 600 605
 Thr Ala Ala Ser Ile Trp Phe His Ile Ile Gly Pro Ser Gly Asn Asp
 610 615 620
 Leu Thr Asn Glu Gly His Asn Phe Ser Ser Val Ser Ser Arg Asn Lys
 625 630 635 640
 Met Phe Val Gln Gly Asn Asn Gly Lys Tyr Val Leu Asn Ile Leu Thr
 645 650 655
 Asp Ser Ile Glu Leu Pro Ser Gly Gln Gln Thr Ile Leu Ile Gln Asn
 660 665 670
 Thr Asn Ser Ser Gln Asp Leu Phe Leu Asp Arg Ile Glu Phe Ile Ser Leu
 675 680 685
 Pro Ser Thr Ser Thr Pro Thr Ser Thr Asn Phe Val Glu Pro Glu Ser
 690 695 700
 Leu Glu Lys Ile Ile Asn Gln Val Asn Gln Leu Phe Ser Ser Ser Ser
 705 710 715 720
 Gln Thr Glu Leu Ala His Thr Val Ser Asp Tyr Lys Ile Asp Gln Val
 725 730 735
 Val Leu Lys Val Asn Ala Leu Ser Asp Asp Val Phe Gly Val Glu Lys
 740 745 750
 Lys Ala Leu Arg Lys Leu Val Asn Gln Ala Lys Gln Leu Ser Lys Ala
 755 760 765

Arg Asn Val Leu Val Gly Gly Asn Phe Glu Lys Gly His Glu Trp Ala
 770 775 780
 Leu Ser Arg Glu Ala Thr Met Val Ala Asn His Glu Leu Phe Lys Gly
 785 790 795 800
 Asp His Leu Leu Leu Pro Pro Pro Thr Leu Tyr Pro Ser Tyr Ala Tyr
 805 810 815
 Gln Lys Ile Asp Glu Ser Lys Leu Lys Ser Asn Thr Arg Tyr Thr Val
 820 825 830
 Ser Gly Phe Ile Ala Gln Ser Glu His Leu Glu Val Val Val Ser Arg
 835 840 845
 Tyr Gly Lys Glu Val His Asp Met Leu Asp Ile Pro Tyr Glu Glu Ala
 850 855 860
 Leu Pro Ile Ser Ser Asp Glu Ser Pro Asn Cys Cys Lys Pro Ala Ala
 865 870 875 880
 Cys Gln Cys Ser Ser Cys Asp Gly Ser Gln Ser Asp Ser His Phe Phe
 885 890 895
 Ser Tyr Ser Ile Asp Val Gly Ser Leu Gln Ser Asp Val Asn Leu Gly
 900 905 910
 Ile Glu Phe Gly Leu Arg Ile Ala Lys Pro Asn Gly Phe Ala Lys Ile
 915 920 925
 Ser Asn Leu Glu Ile Lys Glu Asp Arg Pro Leu Thr Glu Lys Glu Ile
 930 935 940
 Lys Lys Val Gln Arg Lys Glu Gln Lys Trp Lys Lys Ala Phe Asn Gln
 945 950 955 960
 Glu Gln Ala Glu Val Ala Thr Thr Leu Gln Pro Thr Leu Asp Gln Ile
 965 970 975
 Asn Ala Leu Tyr Gln Asn Glu Asp Trp Asn Gly Ser Val His Pro Ala
 980 985 990
 Ser Asp Tyr Gln His Leu Ser Ala Val Val Val Pro Thr Leu Pro Lys
 995 1000 1005
 Gln Arg His Trp Phe Met Glu Gly Arg Glu Gly Glu His Val Val Leu
 1010 1015 1020
 Thr Gln Gln Phe Gln Gln Ala Leu Asp Arg Ala Phe Gln Gln Ile Glu
 1025 1030 1035 1040
 Glu Gln Asn Leu Ile His Asn Gly Asn Leu Ala Asn Gly Leu Thr Asp
 1045 1050 1055

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Trp Thr Val Thr Gly Asp Ala Gln Leu Thr Ile Phe Asp Glu Asp Pro	
1060	1065 1070
Val Leu Glu Leu Ala His Trp Asp Ala Ser Ile Ser Gln Thr Ile Glu	
1075	1080 1085
Ile Met Asp Phe Glu Gly Arg His Arg Ile Gln Thr Ala Cys Thr Trp	
1090	1095 1100
Lys Arg Gln Arg Asn Ser Tyr Arg Ser Thr Trp Arg Lys Arg Leu Glu	
1105	1110 1115 1120
Thr Met Thr Phe Asn Thr Thr Ser Phe Thr Thr Gln Glu Gln Thr Phe	
1125	1130 1135
Tyr Phe Glu Gly Asp Thr Val Asp Val His Val Gln Ser Glu Asn Asn	
1140	1145 1150
Thr Phe Leu Ile Asp Ser Val Glu Leu Ile Glu Ile Ile Glu Glu	
1155	1160 1165

Claims

- 1 1. A polynucleotide sequence from a *Bacillus thuringiensis* isolate selected from the
2 group consisting of PS80JJ1, PS158D5, PS167P, PS169E, PS177F1, PS177G, PS204G4, and
3 PS204G6, which encodes a toxin active against nematodes.
- 1 2. The polynucleotide sequence, according to claim 1, which encodes a toxin of SEQ
2 ID NO. 4.
- 1 3. The polynucleotide sequence, according to claim 2, which is shown in SEQ ID NO.
2 3.
- 1 4. The polynucleotide sequence, according to claim 1, which encodes a toxin of SEQ
2 ID NO. 6.
- 1 5. The polynucleotide sequence, according to claim 4, which is shown in SEQ ID NO.
2 5.
- 1 6. A polynucleotide sequence which encodes a protein toxic to nematodes, wherein said
2 toxin is encoded by a polynucleotide sequence that can be amplified using SEQ ID NO. 1 and
3 SEQ ID NO. 2 as primers.
- 1 7. The polynucleotide sequence, according to claim 6, which encodes a toxin selected
2 from the group consisting of SEQ ID NO. 4 and SEQ ID NO. 6.
- 1 8. The polynucleotide sequence, according to claim 7, which comprises a
2 polynucleotide sequence selected from the group consisting of SEQ ID NO. 3 and SEQ ID NO.
3 5.
- 1 9. A recombinant host transformed by a polynucleotide sequence of claim 1, wherein
2 said recombinant host expresses a nematode-active toxin.
- 1 10. The recombinant host, according to claim 9, wherein said host is a plant.

- 1 11. A nematode-active toxin encoded by a polynucleotide sequence of claim 1.

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Fig. 1A

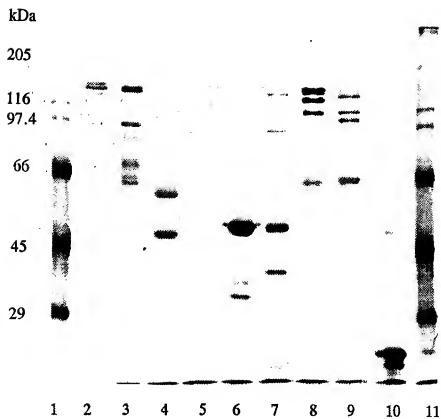


Fig. 1B

